

What is claimed:

1. A cold-adapted equine influenza virus.
2. The virus of Claim 1, wherein said virus replicates in embryonated chicken eggs at a temperature ranging from about 26°C to about 30°C.
- 5 3. The virus of Claim 1, wherein said virus is attenuated.
4. The virus of Claim 1, wherein said virus is temperature sensitive.
5. The virus of Claim 1, wherein said virus replicates in embryonated chicken eggs at a temperature ranging from about 26°C to about 30°C, but does not form plaques in tissue culture cells at a temperature of about 39°C.
- 10 6. The virus of Claim 1, wherein said virus replicates in embryonated chicken eggs at a temperature ranging from about 26°C to about 30°C, but does not form plaques in tissue culture cells at a temperature of about 37°C.
7. The virus of Claim 1, wherein a phenotype comprising a non-permissive temperature of about 39°C is conferred on said virus by at least two mutations in the genome of said virus,
- 15 comprising a first mutation and a second mutation.
8. The virus of Claim 7, wherein said first mutation confers a phenotype on said virus comprising inhibition of plaque formation at a temperature of about 39°C, and wherein said first mutation co-segregates with the segment of said genome comprising the nucleoprotein gene of said virus.
- 20 9. The virus of Claim 7, wherein said second mutation confers a phenotype on said virus comprising inhibition of protein synthesis of said virus at a temperature of about 39°C.
10. The virus of Claim 7, further comprising at least one additional mutation, wherein said additional mutation confers a phenotype comprising a non-permissive temperature of about 37°C on said virus, and wherein said phenotype is selected from the group consisting
- 25 of inhibition of plaque formation at a temperature of about 37°C and inhibition of the expression of the late genes of said virus at a temperature of about 37°C.
11. The virus of Claim 1, wherein said virus is produced by a method comprising the steps of:

- a. passaging a wild-type equine influenza virus; and
- b. selecting viruses that grow at a reduced temperature.

12. The virus of Claim 11, wherein said virus is produced by a method further comprising repetition of said passaging and selection steps one or more times, wherein said reduced  
5 temperature is made progressively lower.

13. The virus of Claim 11, wherein said passaging step is carried out in embryonated chicken eggs.

14. The virus of Claim 11, wherein said virus comprises a dominant interference phenotype.

10 15. The virus of Claim 1, wherein said virus is derived from strain A/equine/Kentucky/1/91 (H3N8).

16. The virus of Claim 1, wherein said virus comprises the identifying phenotypes of a virus selected from the group consisting of: EIV-P821, identified by accession No. ATCC VR-2625 EIV-P824, identified by accession No. ATCC VR-2624 and MSV+5, identified by  
15 accession No. ATCC VR-2627.

17. The virus of Claim 1, wherein said virus is selected from the group consisting of: EIV-P821, identified by accession No. ATCC VR-2625; EIV-P824, identified by accession No. ATCC VR-2624; MSV+5, identified by accession No. ATCC VR-2627; and progeny of any of said viruses having any of said accession numbers.

20 18. A reassortant influenza A virus comprising at least one genome segment of an equine influenza virus generated by cold-adaptation, said equine influenza virus having an identifying phenotype selected from the group consisting of cold-adaptation, temperature sensitivity, dominant interference, and attenuation, wherein said equine influenza virus genome segment confers at least one of said identifying phenotypes to said reassortant virus.

25 19. The reassortant influenza A virus of Claim 18, wherein said virus is produced by a method comprising the steps of:

a. mixing the genome segments of a donor cold-adapted equine influenza virus with the genome segments of a recipient influenza A virus; and

b. selecting a reassortant virus comprising at least one phenotype of said donor equine influenza virus, wherein said phenotype is selected from the group consisting of cold-adaptation, temperature sensitivity, dominant interference, and attenuation.

20. The reassortant influenza A virus of Claim 18, wherein said recipient influenza A virus comprises hemagglutinin and neuraminidase phenotypes different than those of said donor equine influenza virus, and wherein said reassortant virus comprises the hemagglutinin and neuraminidase phenotypes of said recipient virus.

10 21. A therapeutic composition to protect an animal against disease caused by an influenza A virus, comprising a virus selected from the group consisting of: (a) a cold-adapted equine influenza virus; and (b) a reassortant influenza A virus comprising at least one genome segment of an equine influenza virus generated by cold-adaptation, said equine influenza virus having an identifying phenotype selected from the group consisting of cold-adaptation, temperature sensitivity, dominant interference, and attenuation, wherein said equine influenza virus genome segment confers at least one of said identifying phenotypes to said reassortant virus.

22. The therapeutic composition of Claim 21, wherein said therapeutic composition comprises a cold-adapted equine influenza virus, wherein said disease is caused by equine influenza virus, and wherein said therapeutic composition is administered prophylactically to an equid, thereby eliciting an immune response against equine influenza virus in said equid.

23. The therapeutic composition of Claim 21, wherein said therapeutic composition comprises from about  $10^5$  TCID<sub>50</sub> units to about  $10^8$  TCID<sub>50</sub> units of said virus.

24. The therapeutic composition of Claim 21, further comprising an excipient.

25 25. A method to protect an animal against disease caused by an influenza A virus comprising administering to said animal a therapeutic composition comprising a virus selected from the group consisting of: (a) a cold-adapted equine influenza virus; and (b) a

reassortant influenza A virus comprising at least one genome segment of an equine influenza virus generated by cold-adaptation, said equine influenza virus having an identifying phenotype selected from the group consisting of cold-adaptation, temperature sensitivity, dominant interference, and attenuation, wherein said equine influenza virus genome segment  
5 confers at least one of said identifying phenotypes to said reassortant virus.

26. The method of Claim 25, wherein said animal is an equid.

27. The method of Claim 25, wherein said therapeutic composition comprises a cold-adapted equine influenza virus, wherein said disease is caused by equine influenza virus, and wherein said therapeutic composition is administered prophylactically to an equid, thereby  
10 eliciting an immune response against equine influenza virus in said equid.

28. The method of Claim 25, wherein said therapeutic composition is administered to said animal by a route that will allow virus entry into mucosal cells of the upper respiratory tract.

29. A method to produce a cold-adapted equine influenza virus comprising the steps of:  
15           a. passaging a wild-type equine influenza virus; and  
            b. selecting viruses that grow at a reduced temperature.

30. The method of Claim 29, wherein said cold-adapted equine influenza virus is produced by a method further comprising repetition of said passaging and selection steps one or more times, wherein said reduced temperature is made progressively lower.

31. A method to produce a reassortant influenza A virus having at least one genome segment of an equine influenza virus generated by cold-adaptation, said equine influenza virus having an identifying phenotype selected from the group consisting of cold-adaptation, temperature sensitivity, dominant interference, and attenuation, comprising the steps of:

- 5           a.       mixing the genome segments of a donor cold-adapted equine influenza virus with the genome segments of a recipient influenza A virus; and
- b.       selecting reassortant a virus comprising at least one phenotype of said donor equine influenza virus, wherein said phenotype is selected from the group consisting of cold-adaptation, temperature sensitivity, dominant interference, and attenuation.

10   32. The method of claim 31, wherein said recipient influenza A virus comprises hemagglutinin and neuraminidase phenotypes different than those of said donor equine influenza virus, and wherein said reassortant virus comprises the hemagglutinin and neuraminidase phenotypes of said recipient virus.

33. A method to propagate a cold-adapted equine influenza virus comprising a method  
15 selected from the group consisting of propagating said virus in eggs and propagating said virus in tissue culture cells.

34. An isolated equine influenza nucleic acid molecule selected from the group consisting of:

- 20           a.       an isolated nucleic acid molecule that encodes a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:45, SEQ ID NO:48, SEQ ID NO:51, SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:81, SEQ ID NO:86, SEQ ID NO:89, SEQ ID NO:92, SEQ ID NO:95, SEQ ID NO:104 and SEQ ID NO:107; and
- 25           b.       an isolated nucleic acid molecule fully complementary to a nucleic acid molecule of (a).

35. The nucleic acid molecule of Claim 34, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:106 and SEQ ID NO:108 and a nucleic acid molecule comprising a nucleic acid sequence which is fully complementary to any of said nucleic acid sequences.

36. A nucleic acid molecule of Claim 34, wherein said nucleic acid molecule encodes a protein.

37. A nucleic acid molecule of Claim 34, wherein said nucleic acid molecule is selected from the group consisting of Pei<sub>cal</sub>M<sub>252</sub>, Pei<sub>cal</sub>HA<sub>565</sub>, Pei<sub>cal</sub>PB2-N<sub>404</sub>, Pei<sub>cal</sub>PB2-C<sub>398</sub>, Pei<sub>cal</sub>PB2<sub>759</sub>, Pei<sub>cal</sub>NS<sub>230</sub>, Pei<sub>cal</sub>PB1-N<sub>395</sub>, Pei<sub>cal</sub>PA-C<sub>390</sub>, Pei<sub>cal</sub>PB1-C<sub>396</sub>, Pei<sub>ca2</sub>PB1-C<sub>396</sub> and Pei<sub>cal</sub>PB1<sub>757</sub>.

38. An isolated equine influenza protein that comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:45, SEQ ID NO:48, SEQ ID NO:51, SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:81, SEQ ID NO:86, SEQ ID NO:89, SEQ ID NO:92, SEQ ID NO:95, SEQ ID NO:104 and SEQ ID NO:107.

39. The protein of Claim 38, wherein said protein is encoded by a nucleic acid molecule selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID

NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12,SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:25 SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:59,SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:106 and SEQ ID NO:108.

40. An isolated virus comprising a nucleic acid molecule that encodes a protein selected from the group consisting SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:45, SEQ ID NO:48, SEQ ID NO:51, SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:81, SEQ ID NO:86, SEQ ID NO:89, SEQ ID NO:92, SEQ ID NO:95, SEQ ID NO:104 and SEQ ID NO:107.

41. The virus of Claim 40, wherein said virus comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12,SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:25 SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:59,SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:106 and SEQ ID NO:108.

42. The virus of Claim 40, wherein said virus is selected from the group consisting of equine influenza virus and a reassortant influenza virus.

43. The virus of Claim 1, wherein said virus comprises a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6,  
5 SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:106 and SEQ ID NO:108.

44. The virus of Claim 1, wherein said virus encodes a protein selected from the group  
10 consisting of SEQ ID NO:5, SEQ ID NO:11, SEQ ID NO:17, SEQ ID NO:24, SEQ ID NO:48, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:81, SEQ ID NO:92, SEQ ID NO:95 and SEQ ID NO:107.

45. The virus of Claim 18, wherein said virus comprises a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6,  
15 SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:106 and SEQ ID NO:108.

46. The virus of Claim 18, wherein said virus encodes a protein selected from the group  
20 consisting of SEQ ID NO:5, SEQ ID NO:11, SEQ ID NO:17, SEQ ID NO:24, SEQ ID NO:48, SEQ ID NO:58, SEQ ID NO:69, SEQ ID NO:81, SEQ ID NO:92, SEQ ID NO:95 and SEQ ID NO:107.